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INSERTION/DELETION-RELATED POLYMORPHISMS IN THE HUMAN T CELL RECEPTOR β GENE COMPLEX

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The TCR, a heterodimer comprised of an α and β chain, mediates the corecognition of antigen and MHC molecules by T cells. The α and β chains of the TCR are encoded in gene complexes that include segments corresponding to the V, J, D, and C regions of the receptor chains (1–3). Diversity, which characterizes T cell responses, is generated by rearrangement of TCR gene segments in the course of T cell maturation (4). Somatic mutation appears to play little or no role in the diversity of the TCR, thus enhancing the importance of polymorphism in the germline genes (5–7). Genetic polymorphism reported to date in human TCR-β genes has been limited relative to that described for some mouse strains in which significant deletions occur within the TCR-β complex (8–10). In the current report, we show that areas of insertion/deletion are likewise present within the human TCR-β gene complex.

Materials and Methods

Preparation of λ Ladder. λ DNA NM1149 (42 kb) or clts857Sam7 (Bethesda Research Laboratories, Gaithersburg, MD) was heated at 65°C for 5 min and then held on ice for 5 min. The DNA solution was diluted to 50 μg/ml in 10 mM Tris/HCl, 10 mM EDTA, pH 8 (TE)[1], and mixed gently with an equal volume of 1% low-melting agarose (International Biotechnologies, Inc.) in TE buffer pre-equilibrated at 50°C. 2 μg of DNA was added per well. The plastic cast was kept on ice for 15 min in order to allow the agarose blocks to solidify. The agarose blocks were removed from the plastic cast and incubated at 37°C in 3× SSC/10 mM EDTA for 3 h. The inserts were washed twice in TE buffer and stored at 4°C.

Preparation of DNA in Agarose Blocks. This procedure works equally well for PBL isolated by density gradient centrifugation as for EBV-transformed lymphoblastoid cells. Agarose blocks containing human DNA were prepared as described by Van Ommen and Verkerk (11). 10^7 cells were washed twice in HBSS and resuspended in 0.125 M EDTA at a concentration of 1.7×10^7 cells/ml. A 1% solution of low-melting temperature agarose (IBI) in 0.125 M EDTA was melted, equilibrated at 50°C, and mixed with an equal volume of the cell suspension at room temperature. The mixture was quickly poured in a 12-well plastic cast sealed by tape on one side and left on ice for 20 min. Agarose inserts were removed from casts using a Pasteur pipet and incubated for 2 d at 50°C in 10 ml of prewarmed 0.5 M EDTA (pH 8), 1% sodium

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[1] *Abbreviations used in this paper:* F, father; IDRP, insertion/deletion-related polymorphisms; M, mother; PFGE, pulsed-field gel electrophoresis; TE, 10 mm Tris/HCl, 10 mm EDTA, pH 8.

sarkosyl, and freshly added 2 mg/ml proteinase K (Bethesda Research Laboratories). Individual blocks, each containing 5 μg of DNA, were stored at 4°C.

Digestion of DNA in Agarose Blocks. Each block was incubated twice at 50°C in 5 ml of TE buffer containing 0.4 mg/ml PMSF (Bethesda Research Laboratories) and once in 5 ml of TE at 50°C; incubations were for 30 min each. Blocks were equilibrated in 1× digestion buffer (New England Biolabs, Beverly, MA) by five successive 10-min incubations in 5 ml of buffer, followed by a single 5-min incubation in 1 ml of buffer supplemented with 100 μg/ml of BSA (Bethesda Research Laboratories). The digestion was carried out overnight in a total volume of 200 μl using 10 U of enzyme/μg of DNA added in two equal portions. The digest reaction was covered with a thin layer of parafin oil.

For double digest experiments, the initial digest was performed as above; the agarose blocks were then equilibrated with the new buffer and the second digest was performed. After digestion, the agarose blocks were incubated with 1 ml of 0.5 M EDTA, 1% sarkosyl, 0.5 mg of proteinase K/ml for 3 h at 50°C.

Gel Electrophoresis Conditions. The Pulsaphor equipment (Pharmacia Fine Chemicals, Piscataway, NJ) was used in all experiments. Digested samples were equilibrated in 10 ml of TE buffer for 30 min before loading onto a 1% agarose gel (Bethesda Research Laboratories) in 1× 100 mM Tris/100 mM boric acid/2 mM EDTA. The gel was run for 44 h at 330 V with a pulse time (unless otherwise stated) of 20 s, and a buffer temperature equilibrated at 12°C. Good resolution of restriction fragments between 40 and 350 kb was obtained under these conditions.

Transfer and Hybridization. Ethidium bromide–stained gels were treated with 0.25 N HCl for 30 min. The DNA was then denatured, neutralized, and transferred to nylon membranes (Hybond-N; Amersham Corp., Arlington Heights, IL) bathed in 10× SSC according to the manufacturer's recommendations. The filter was baked for 2 h at 80°C. DNA probes were labeled with α-[^{32}P]dCTP by the method of random priming (12) to a specific activity of 10^9 cpm/μg. Filters were prehybridized at 42°C for 4 h in 40% formamide, 5× SSC, 5× Denhardt's, 50 mM phosphate buffer, pH 7, 0.5% SDS, and 100 μg of deproteinased and denatured salmon sperm DNA/ml. Filters were hybridized for 20 h at 42°C with 2–3 × 10^6 cpm probe/ml in a 10-ml solution of 40% formamide, 5× SSC, 5× Denhardt's, 50 mM phosphate, pH 7, 0.5% SDS, and 10% dextran sulfate. Filters were washed twice for 20 min each at 50°C with 0.4× SSC, 0.1% SDS, and exposed humid to XAR-5 films at −70°C with an intensifying screen for 24–48 h.

Results and Discussion

DNA samples from unrelated persons were digested with Sfi I, separated by pulse field gel electrophoresis (PFGE), and the blots hybridized with probes for the Vβ1 (13), Vβ6 (14), muC5V (murine Vβ probe designated C5) (15, 16), Vβ8.1 (17), Vβ11 (14), Vβ12 (14), and Cβ2 (18) gene segments. The four Sfi I fragments containing clusters of V region gene segments previously identified by Lai et al. (19) could be confirmed, and it was possible to further extend the restriction map of the TCR-β complex. Of greater interest was the identification of two Sfi I polymorphisms, the first a 300/330-kb fragment detected with probes Vβ1, Vβ6, muC5V, or Vβ12, and the second a 130/145-kb fragment detected with Cβ2.

In Fig. 1, segregation of 300/330 and 130/145 Sfi I polymorphic fragments in four healthy Caucasian families is shown; the 300/330 fragments hybridize with the Vβ1 probe and the 130/145 fragments with the Cβ probe. In Fig. 1 *A*, father (F) is homozygous for the 330-kb fragment and mother (M) is heterozygous; children S1–S4 are heterozygous, having each inherited the maternal 300-kb fragment and a paternal 330-kb fragment, while S5, S6, and S7 have inherited the maternal 330-kb fragment and a paternal 330-kb DNA fragment. In Fig. 1 *B*, F is homozygous for the 330-kb fragment and M has both the 300- and the 330-kb fragments; S1–S4 have each in-

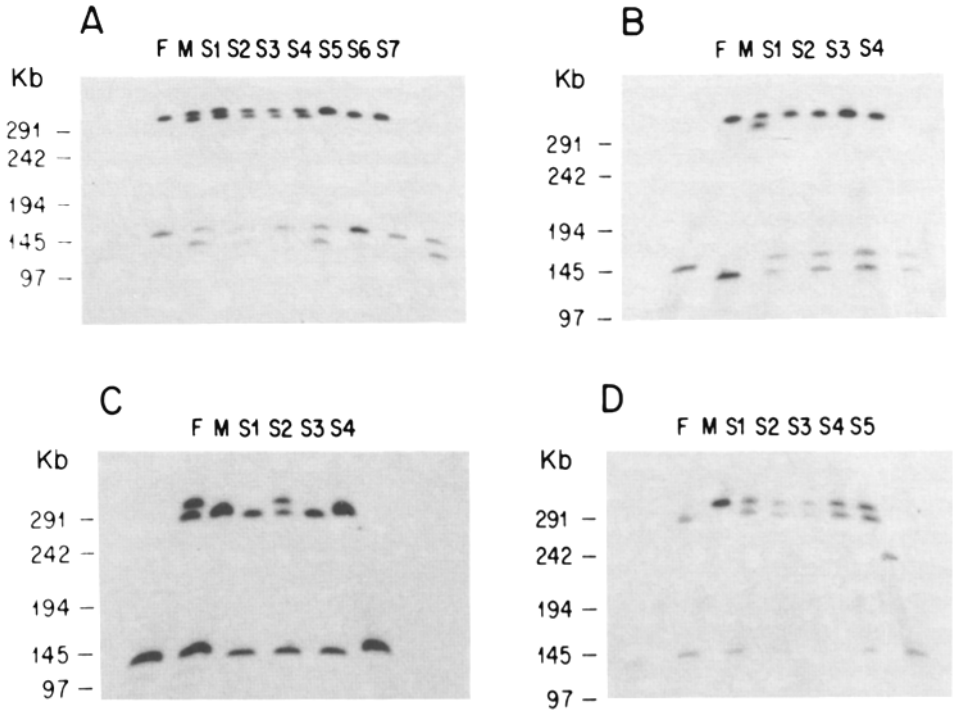

FIGURE 1. Segregation of 300/330 and 130/145 Sfi I polymorphic fragments in four healthy Caucasian families. In repeated experiments, whole PBL or EBV-transformed cell lines derived from the same individual resulted in identical IDRP patterns by PFGE (data not shown). PBL were the source of DNA in *B* and *D*, and EBV lines were used in *A* and *C*. See text for details.

herited the 330-kb maternal allele and one of the 330-kb paternal alleles. In Fig. 1 *C*, F is heterozygous and M is homozygous for the 300-kb fragment; their children carry either the 300/300 (S1, S3, and S4) or the 300/330 (S2) haplotypes. In Fig. 1 *D*, F is homozygous for the 300-kb fragment and M is homozygous for the 330/kb fragment; all siblings (S1–S5) have inherited one allelic fragment from each parent and are heterozygous for the 300/330 polymorphism.

With regard to the 130/145 polymorphism, in Fig. 1 *A*, F is heterozygous and M homozygous for the 145-kb fragment. S3, S5, and S6 have inherited a 145-kb allele from each parent and are thus homozygous; the remaining children are heterozygous, having inherited the 130-kb fragment from F and one of the two 145-kb maternal allelic fragments. In Fig. 1 *B*, both parents are homozygous, F for the 145-kb fragment, and M for the 130-kb fragment; all children are heterozygous. In Fig. 1, *C* and *D*, both parents and all children are homozygous for the 145-kb fragment.

The finding that both the 300/330-kb and 130/145-kb alleles segregate within families indicates that they are neither artifacts of incomplete digestion nor due to somatic mutation and/or rearrangement of germline DNA.

The polymorphisms observed by PFGE could have been generated either by point mutations that create or delete Sfi I sites or by insertion/deletion events. To distinguish between these possibilities, DNA samples representing different combinations

of the Sfi I polymorphisms were digested with Sal I and hybridized with Cβ2 or Vβ6 probes (Fig. 2). If the polymorphisms are due to point mutations, then Sal I fragments that overlap polymorphic Sfi I fragments should be nonpolymorphic; if, on the other hand, these polymorphisms result from an insertion or a deletion of a DNA fragment, then corresponding size variations of the Sal I fragments should be observed. Hybridization with Cβ2 revealed polymorphic Sal I fragments of 565/580-kb that correlated precisely with the Sfi I 130/145-kb polymorphism (Fig. 2 *A*). When Sal I digests were hybridized with Vβ6, an additional 510/540-kb Sal I polymorphism was found that segregated in a concordant fashion with the 300/330 Sfi I RFLP (Fig. 2 *B*). These results indicate that the 300/330 and 130/145 Sfi I polymorphisms result from insertion or deletion of DNA fragments of ~30 and ~15 kb, respectively, and may be properly designated as insertion/deletion-related polymorphisms (IDRP).

The regions including the IDRP within the TCR-β complex were localized in double digest experiments (Fig. 3). DNA samples representing different Sfi I types were digested with Sfi I alone or with both Sfi I and Sal I, and hybridized either with Vβ6, Cβ2, or muC5V probes. An invariant 110-kb fragment along with the polymorphic 300/330-kb fragment was found in all Sfi I-digested samples hybridized with Vβ6 (Fig. 3 *A*). Double digestion with Sal I and Sfi I generated three additional fragments of 50, 70/100, and 180 kb, suggesting, as reported (19), that two Sal I sites are located within the 300/330-kb fragment. Hybridizing bands corresponding to partial Sal I digests revealed that the 180-kb Sal I/Sal I fragment is flanked by the 50-kb and the polymorphic 70/100-kb fragments (Fig. 3 *A*). The orientation of these Sal I/Sfi I fragments (Fig. 3 *B*) could be determined by examining the hybridization pattern obtained with muC5V as probe. The muC5V probe hybridized exclusively to the 300/330-kb Sfi I fragment (Fig. 3 *B*). In addition, concordant polymorphisms of muC5V and Vβ8.1 hybridizing genes have been observed in Bam HI-digested DNA samples (13). In one allelic configuration both probes hybridize

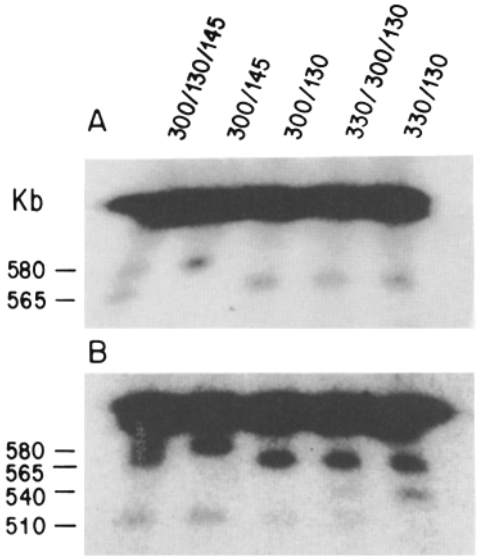

FIGURE 2. DNA obtained from PBL from individuals with the 300/130/145-, 300/145-, 300/130-, 300/330/130-, and 330/130-kb Sfi I alleles were digested with Sal I, the fragments separated with a pulse time of 42 (*A*) or 40 s (*B*), and the gel hybridized with cβ2 (*A*) or Vβ6 (*B*). Filters were exposed for 5 d.

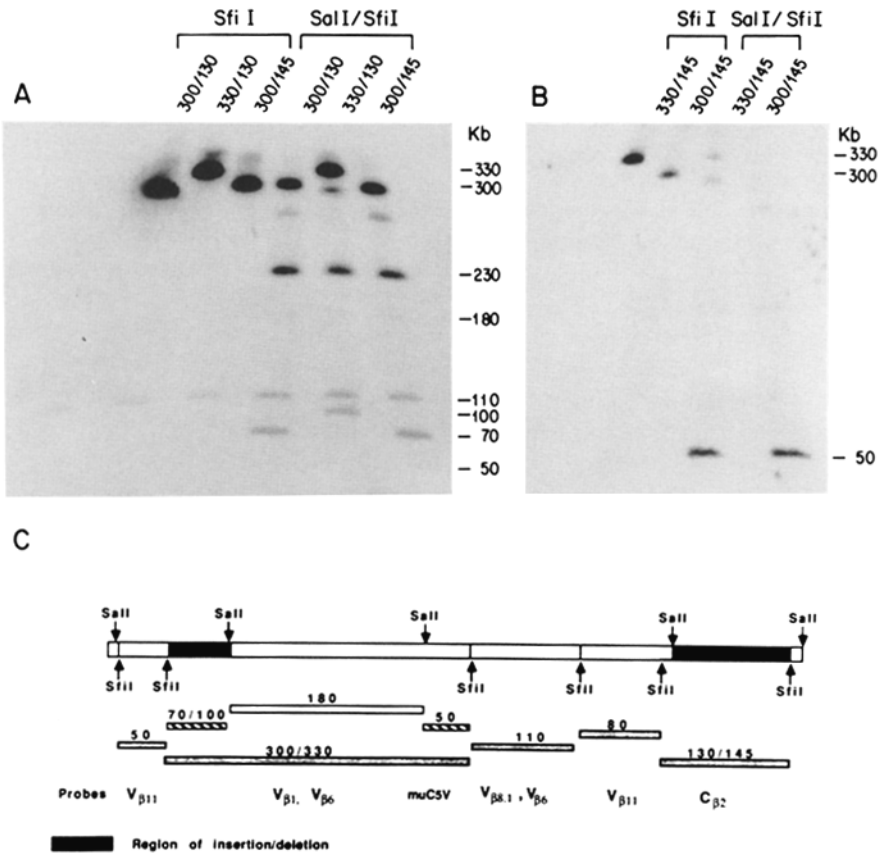

FIGURE 3. (*A*) DNA obtained from PBL with the 300/130-, 330/130-, and 300/145-kb Sfi I RFLP were digested with Sfi I alone or with both Sfi I and Sal I enzymes and hybridized to a Vβ6 probe. (*B*) DNA samples with the 300/130- or 330/130-kb Sfi I RFLP were digested as in *A* and hybridized with muC5V. (*C*) Representation of TCR-β gene complex in man showing areas in which size polymorphisms have been found. Sal I fragments are designated by open bars, Sfi I fragments by shaded bars, and Sfi I/Sal I fragments by striped bars. Fragment sizes in kilobases are indicated above the bars, polymorphic fragments are shown with both observed sizes. Regions of probe hybridization are indicated at the bottom of the figure.

with a fragment of 24 kb; in the other allelic form the muC5V probe hybridizes with a 22-kb fragment and the Vβ8.1 probe with a 2.2-kb fragment, localizing a polymorphic Bam HI restriction site between the two genes. Double digestion with Bam HI and Sfi I revealed an invariant Sfi I site within the 22/24-kb Bam HI fragment (data not shown). Because Vβ8.1 has been localized to the 5′ end of the 110-kb Sfi I fragment (19, 20) that is located immediately 3′ to the 300/330-kb Sfi I fragment, hybridization studies localized muC5V to the 3′ end of the 300/330-kb fragment. In double digest experiments, muC5V hybridized exclusively with the 50-kb Sal I/Sfi I fragment, placing this fragment at the 3′ end and the polymorphic fragment at the 5′ end of the 300/330-kb fragment (Fig. 3, *B* and *C*). This reorientation of the Sal I sites with respect to the current map of the TCR-β (19) places this insertion/deletion locus 5′ to most members of the Vβ6 family. Using a similar approach,

the 130/145-kb Sfi I polymorphism detected using the Cβ2 probe could be localized (Fig. 3 *C*).

Haplotype frequencies for the 300/330- and 130/145-kb alleles were determined in 50 unrelated healthy Caucasian individuals (Table I). It was possible to type all individuals for the two IDRP, except one in which fragments of 270 and 330 kb were observed. Allele frequencies for the 300/330 polymorphisms were estimated to be 0.61 for the 300-kb fragment and 0.37 for the 330-kb fragment. For the 130/145 polymorphism, the allelic frequency was 0.46 for the 130-kb fragment and 0.54 for the 145-kb fragment. All possible combinations between the two allelic groups were observed.

It is not known whether sequences comprising the regions of insertion/deletion encode expressed V gene segments. In one study of the germline Vβ repertoire in 100 unrelated humans utilizing probes that recognize an estimated 48 Vβ segments, an apparent deletion of one member of the Vβ6 family (Vβ6.2) was detected in a single individual (21). As the 300/330 Sfi I IDRP occurred frequently in unrelated individuals (6/50 individuals were homozygous for the 300-kb fragment), it is unlikely that the rare deletion identified in that earlier study corresponds to this polymorphism. Furthermore, conventional southern blot analysis of Eco RI- and Hind III-digested genomic DNA hybridized to the Vβ6 probe under conditions of low stringency did not detect the presence of bands that were absent in homozygotes for the 300-kb Sfi I fragment (data not shown).

The presence of the 300/330 and 130/145 Sfi I polymorphisms should prove useful in genetic studies of diseases associated with abnormalities of T cell function, including autoimmune disorders. As clonal T cell responses are characterized by allelic exclusion of TCR genes, the parental origin of the TCR gene rearranged in individual T cell responses can also be defined by this method. The observation that regions of insertion/deletion are commonly found within the human TCR-β adds a new dimension to the genetic variability of this gene complex. The observation of an additional polymorphism in one of 50 individuals sampled suggests that study of larger and more diverse human populations may uncover further examples of IDRP. Previous studies of haplotype heterogeneity have suggested that frequent crossover events have contributed to the polymorphism of the TCR-β genes (13). While it is tempting to speculate that the presently observed areas of insertion/deletion have resulted from nonhomologous crossover events in this complex, insertions mediated by retroviruses or by transposons cannot be excluded. Irrespective of the mechanism whereby the two IDRP were generated, their widespread occurrence in all

TABLE I

Frequencies for the 300/330- and 130/145-kb Haplotypes

	Polymorphisms		
Polymorphisms	130	145	130/145
300	0.12	0.12	0.18
330	0.04	0.06	0.06
300/330	0.10	0.16	0.12

Data shown are frequencies of the 300/330- and 130/145-kb Sfi I polymorphisms in 50 unrelated healthy Caucasian individuals. DNA was digested from PBL (n = 8) or from EBV cell lines (n = 42).

possible combinations argues that the causative events occurred quite early in the evolutionary history of man.

Summary

Insertion/deletion related polymorphisms (IDRP) involving stretches of 15–30 kb within the human TCR-β gene complex were revealed by pulse-field gel electrophoresis. Two independent IDRP systems were detected by analysis of Sfi I- and Sal I-digested human DNA samples using probes for TCR C and V region gene segments. The allelic nature of these systems was verified in family studies, and mapping data allowed localization of one area of insertion/deletion among the V gene segments and the other near the C region genes. All but one of 50 individuals tested could be typed for the two allelic systems, and gene frequencies for the two allelic forms were 0.37/0.61 and 0.46/0.54, indicating that these polymorphisms are widespread.

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